APPENDIX of mtDNA COI barcode data from Monash Uni. genomics lab, Malaysia plus information on first Japanese record of *Lumbricus terrestris* L., 1758.

By Dr R.J. Blakemore Zama, Japan, 9th Feb,. 2022.

#6.1 – *D. veneta* Zama sample was not extracted nor amplified at the first attempt.

#6.2 – *Dendrobaena veneta* Japanese specimen (KPM-NJL 78) from compost of Zama garden (collected RJB Nov. 2020); GenBank OM303091 second attempt:

GCCCCACCCTTTACTTCATTTTAGGTATTTGAGCCGGAATAGTGGGTGCTGGAATA
AGACTACTCATTCGAATTGAATTAAGACAACCTGGTGCCTTTTTAGGCAGAGATCA
ATTATACAACACCATTGTTACAGCACACGCCTTTGTTATAATTTTCTTCTTGGTGAT
ACCAGTATTTATTGGGGGATTCGGAAACTGACTCCTCCCCCTAATACTAGGGGCC
CCAGATATAGCATTTCCCCGACTAAACAACATAAGGTTTTGATTACTACCACCCTC
ACTAATTCTATTAGTTTCTTCTGCTGCCGTAGAAAAAGGTGCTGGCACCGGCTGA
ACCGTCTACCCTCCCA

BLASTn: 99.00% *Dendrobaena veneta* KP420567 from "*Netherlands: vermiculture*"; 98.84% *D. veneta* MF417020 from Crete; 98.84% *D. veneta* KT823850 from Turkey; 97.82% "*Eisenia zebra*" FJ214229 from "*USA: Florida*" (a long time junior synonym of *D. veneta*); 97.66% *D. veneta* KC788612 from Verdun, France. I.e., agrees ~98–100% with diagnosis as *D. veneta* from Japan.

Notes: 97.14% *D. veneta* KM823561 from Poland differs by 2.86% (i.e. tolerably the same taxon as less than ~3% difference), whilst 81.85% for "Dendrobaena veneta" MT968746 from Iran differs >18% as a mistake (its BLAST result gave no matches closer than 87% so the actual species remains unidentified at this time).

The closest match of 99% to a Netherlands worm may be pertinent indication of a possible initial source (via USA?) of the Japanese specimens since a company there (https://dutchredworms.com/company) says: "*Our goal is to supply the highest quality European nightcrawlers, Eisenia hortensis or Dendrobaena veneta, live bait to our customers around the world*".

#AT1 – Dry worm from Zama garden (collected by Aisla B. Tansy 11th Nov., 2018) (Accession No. KPM-NJD 99):

CCACTCTATACTTCATTTTAGGAATTTGAGCCGGAATAATTGGGGCTGGAATAAGT
CTTCTTATTCGAATCGAATTAAGACAACCTGGGTCATTCCTGGGAAGTGACCAAC

TTTATAACACAATTGTAACAGCACATGCATTCTTAATAATTTTTTTCTAGTAATAC
CAGTATTTATTGGTGGTTTTGGTAATTGACTATTACCACTTATATTGGGGACTCCA
GATATAGCATTTCCACGCCTAAATAATATAAGATTCTGACTATTACCCCCCTCACTC
ATCTTACTAGTCTCTTCTGCAGCAGTGGAAAAAGGTGCAGGAACAGGATGAACAG
TTTACCCACCACTAGCAAGTAACATTGCGCATGCTGGACCATCAGTAGATCTGGC
AATTTTCTCACTACACTTAGCAGGGGCATCATCAATTCTAGGTGCAATCAATTTTA
TTACTACAGTAATTAATATACGATGATCAGGCCTACGACTAGAACGAATTCCCCTA
TTTGTATGAGCAGTAGTAATTACCGTAGTTCTACTACTTCTATCATTACCAGTACTA
GCCGGTGCTATCACAATACTATTAACAGATCGAAACCTTAATACATCATTCTTTGAT
CCAGCAGGAGGGGGAGACCCAATTTTATATCAACACCTATTTTGATTTTTTGGTCA
CCCTGAAGTTTAGTCATACTGGTTTCCCTGA
BLASTn: 100% *Amynthas corticis* (Kinberg, 1867), e.g. MH845450 from China or AMNZ86033 from NZ identified by current author. An unremarkable record for Japan, this result yet shows DNA can classify an otherwise unidentifiable specimen.

It was not possible to upload this sequence to Genbank as it automatically generated an error message.

To this Lim Shu-Yong in January, 2022 replied:
"*Hi Robert,*

We used the NCBI vector contamination site to check for possible contamination and had trimmed off the vector contamination in K5.2 and K7. As for the other errors, which are the stop codon and frameshift, we can't do much as this is the biological nature of the sample.

I have attached the trimmed sequences in this email.

Regards,
Shu Yong, Lim
Assistant Manager, Research and Technical Services
Genomics Facility"

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>#6.2 Dendrobaena veneta (voucher Odawara KPM-NJL 78) cytochrome oxidase subunit I
(COI) gene, partial cds; mitochondrial.
GCCCCACCCTTTACTTCATTTTAGGTATTTGAGCCGGAATAGTGGGTGCTGGAATAAGACTACTCATTCGAATTGAATTAAGACAA
CCTGGTGCCTTTTTAGGCAGAGATCAATTATACAACACCATTGTTACAGCACACGCCTTTGTTATAATTTTCTTCTTGGTGATACC
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AGTATTTATTGGGGGATTCGGAAACTGACTCCTCCCCCTAATACTAGGGGCCCCAGATATAGCATTTCCCCGACTAAACAACATAA
GGTTTTGATTACTACCACCCTCACTAATTCTATTAGTTTCTTCTGCTGCCGTAGAAAAAGGTGCTGGCACCGGCTGAACCGTCTAC
CCTCCCA
>#AT1 Amynthas corticis (voucher Odawara KPM-NJD 99)cytochrome oxidase subunit I (COI)
gene, partial cds; mitochondrial.
CCACTCTATACTTCATTTTAGGAATTTGAGCCGGAATAATTGGGGCTGGAATAAGTCTTCTTATTCGAATCGAATTAAGACAACCT
GGGTCATTCCTGGGAAGTGACCAACTTTATAACACAATTGTAACAGCACATGCATTCTTAATAATTTTTTTTCTAGTAATACCAGT
ATTTATTGGTGGTTTTGGTAATTGACTATTACCACTTATATTGGGGACTCCAGATATAGCATTTCCACGCCTAAATAATATAAGAT
TCTGACTATTACCCCCCTCACTCATCTTACTAGTCTCTTCTGCAGCAGTGGAAAAAGGTGCAGGAACAGGATGAACAGTTTACCCA
CCACTAGCAAGTAACATTGCGCATGCTGGACCATCAGTAGATCTGGCAATTTTCTCACTACACTTAGCAGGGGCATCATCAATTCT
AGGTGCAATCAATTTTATTACTACAGTAATTAATATACGATGATCAGGCCTACGACTAGAACGAATTCCCCTATTTGTATGAGCAG
TAGTAATTACCGTAGTTCTACTACTTCTATCATTACCAGTACTAGCCGGTGCTATCACAATACTATTAACAGATCGAAACCTTAAT
ACATCATTCTTTGATCCAGCAGGAGGGGGAGACCCAATTTTATATCAACACCTATTTTGATTTTTTGGTCACCCTGAAGTTTAGTC
ATACTGGTTTCCCTGA
>K5.2 Amynthas kume (Holotype LBM FY2015-11-1)cytochrome oxidase subunit I (COI) gene,
partial cds; mitochondrial.
TAATTGGCGATACTGCCATTGCTGACATCAGTAGACCTTGCAATTTTTTCGCTACATTTAGCGGGAGCCTCTTCAATTTTAGGAGC
CATTAATTTTATCACCACCGTGATCAACATACGTTGATCGGGACTACGTCTAGAACGAATCCCACTATTTGTATGGGCAGTGGTAA
TTACCGTAGTACTACTTCTTCTATCCCTACCAGTTTTAGCAGGGGCCATTACAATACTATTAACAGACCGAAACCTAAATACTTCA
TTCTTTGACCCAGCTGGCGGGGGAGACCCAATTCTATACCAACATCTATTTTGATTTTTTGGTCACCCTGAAGTT
>K7 Metaphire kinki (Holotype LBM FY2015-8-1)cytochrome oxidase subunit I (COI) gene,
partial cds; mitochondrial.
TACCACTCTTTACTTCTCCTAGGTATCTGACCCGGGATAATTGGAGCTGGTATAAGACTATTAATTCGAATTGAATTAAGACAACC
GGGCTCATTTTTAGGAAGTGATCAACTATATAACACAATTGTAACCGCACACGCCTTTCTTATAATCTTCTTCTTAGTAATACCAG
TATTTATTGGAGGATTTGGAAACTGGTTGTTACCTTTAATACTTGGAACACCAGACATAGCTTTTCCACGACTTAATAACATAAGA
TTTTGACTACTACCTCCATCGCTTATTCTATTAGTTTCATCTGCTGCAGTAGAAAAAGGAGCTGGAACAGGATGAACAGTATACCC
GCCACTAGCTAGTAATATCGCACATGCTGGACCCTCCGTAGATCTTGCTATTTTCTCGCTACATTTAGCTGGAGCCTCTTCAATTC
TTGGTGCCATTAATTTCATTACCACAGTAGTTAATATACGATGATCAGGACTACCATTAGAACGAATTCCACTATTTGTCTGAGCC
GTATTAATTACCGTATTACTACTACTACTATCCCTACCAGTACTCGCAGGTGCTATTACAATACTACTAACAGACCGTAACCTAAA
TACATCCTTCTTCGACCCTGCAAGAGGACGATATCCTATCCTATATCTACACCTATCTTTG
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Attempts to upload these sequences were also blocked by Genbank error messages.

Regarding first Japanese record of *Lumbricus terrestris* L. 1758

Initial preliminary report of *Lumbricus terrestris* from Japan is by Blakemore (2021 – https://vermecology.wordpress.com/2021/12/31/lterrestris-in-japan/) now supported with specimens deposited in KPMNH, Odawara. Its full taxonomic description based upon the restored British Museum Neotype plus many other specimens, its ecology and global distribution (with an earlier new report from Tasmania and thus for Australia) are provided in Blakemore (1997, 2000, 2013, 2020). Confirmation of this "classical" cosmopolitan as a new exotic species from Japan is thus also a new record for East Asia (Figs. 7–8).

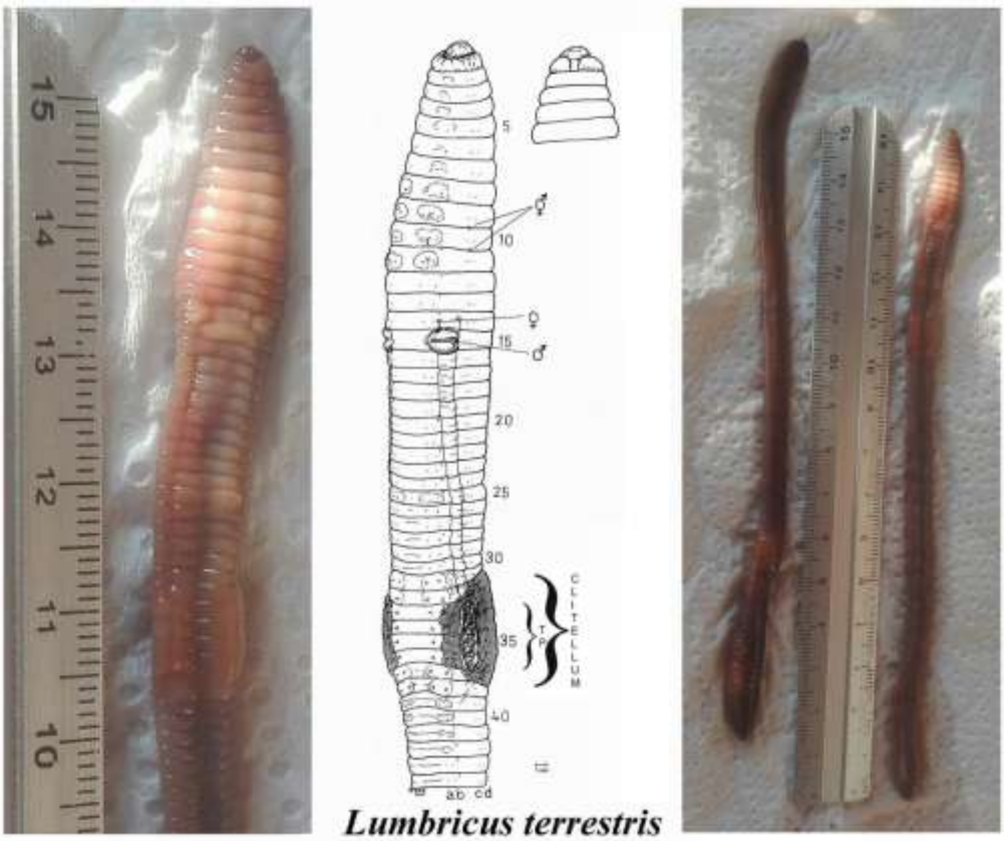
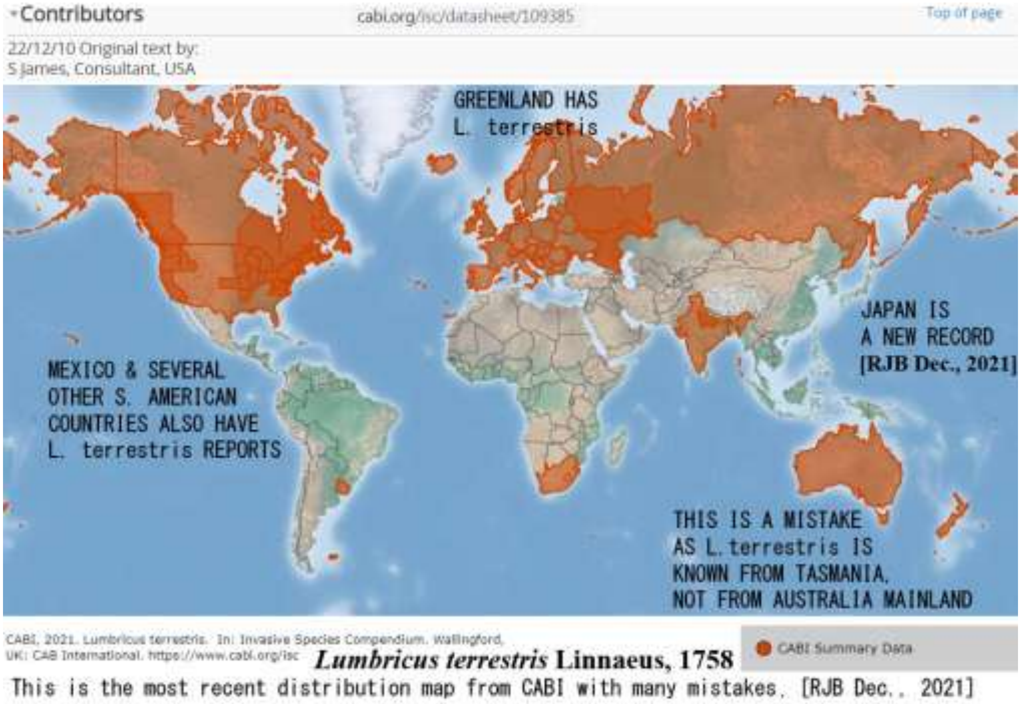


Figure 7. *Lumbricus terrestris* Linnaeus, 1758 diagram (from Blakemore 2000, 2020) compared to specimens deposited in Odawara Museum in 2021 (KPM-NJL 79-80).



CABI, 2021. Lumbricus terrestris. In: Invasive Species Compendium. Wallingford, UK: CAB International. https://www.cabi.org/isc

Lumbricus terrestris **Linnaeus, 1758**

This is the most recent distribution map from CABI with many mistakes. [RJB Dec., 2021]

Figure 8. CABI's global distribution of European *L. terrestris* requires updating.

References

Blakemore, R.J. (2000). *Tasmanian Earthworms*. CD-ROM Monograph with Review of World Families. VermEcology, Kippax ACT 2615. Australia, December, 2000. Pp. 800 including 222 figs. ISBN 0-646-41088-1. Online: https://archive.org/details/tasmanianearthw00blak ; https://www.biodiversitylibrary.org/item/287911#page/1/mode/1up .

Blakemore, R.J. (2003). Japanese Earthworms (Annelida : Oligochaeta): a Review and Checklist of Species. *Organisms, Diversity and E*volution, **3(3):** 1-43; 241-244. Online - http://www.senckenberg.uni-frankfurt.de/odes/03-11.pdf.

Blakemore, R.J. (2015). Eco-taxonomic profile of iconic vermicomposter – the 'African Nightcrawler' earthworm, *Eudrilus eugeniae* (Kinberg, 1867). *African Invertebrates*. **56(3):** 527-548. DOI: https://doi.org/10.5733/afin.056.0302.

Blakemore, R.J. 2018b. Critical Decline of Earthworms from Organic Origins under Intensive, Humic SOM-Depleting Agriculture. *Soil Systems*, **2(2):** 33. [http://www.mdpi.com/2571-8789/2/2/33].

Blakemore, R.J. (2019). Extinction of Japan's first formally described earthworm *Amynthas japonicus* (Horst, 1883). *Bulletin of Kanagawa Prefectural Museum* (*Natural Sciences*). **48:** 55-60. https://nh.kanagawa-museum.jp/www/contents/1600214845212/simple/bull48_55_60_blakemore.pdf.

Blakemore, R.J. (2020). *Cosmopolitan Earthworms.* (6th Amended Edition). VermEcology, Yokohama, Japan, December, 2000. Pp 1,250 + 150 figs.

Blakemore, R.J. (2021). Definitive checklist & IUCN Redlist for Japanese native & exotic earthworms. VermEcology, Zama, Japan. Online – https://vermecology.wordpress.com/2020/12/24/earthworms-of-japan/comment-page-1/.

Blakemore, R.J., Kaneko, N., *et al.* (2007). Alien earthworms in the Asia/Pacific region with a checklist of species and the first records of *Eukerria saltensis* (Oligochaeta : Ocnerodrilidae) and *Eiseniella tetraedra* (Lumbricidae) from Japan, and *Pontoscolex corethrurus* (Glossoscolecidae) from Okinawa. In: Koike, F., *et al.* (eds.), *Assessment and Control of Biological Invasion Risks*. IUCN, Gland, Switzerland and Cambridge, UK, and Shoukadoh Book Sellers, Kyoto, Japan, 2007. Pp 173-181. Online: http://vege1.kan.ynu.ac.jp/isp/pdf/IAS_risk.pdf http://www.iucn.org/dbtw-wpd/edocs/2006-061.pdf.

Briones, M.J.I., Moran, P., Posada, D. (2009), Are the sexual, somatic and genetic characters enough to solve nomenclatural problems in lumbricid taxonomy? *Soil Biology & Biochemistry* . **41:** 2257–2271. DOI: 10.1016/j.soilbio.2009.07.008 ; www.researchgate.net/publication/222896850_Are_the_sexual_somatic_and_genetic_characters_enough_to_solve_nomenclatural_problems_in_lumbricid_taxonomy.

Easton, E.G. 1981. Japanese earthworms: a synopsis of the Megadrile species (Oligochaeta). *Bulletin of the British Museum* (*Natural History, Zoology*), **40(2):** 33-65.

Fiołka, M.J., Rzymowska, J., *et al.* (2019). Antitumor activity and apoptotic action of coelomic fluid from the earthworm *Dendrobaena veneta* against A549 human lung cancer cells. *APMIS.* **127(6):** 435-448. DOI: 10.1111/apm.12941.

Kale, R.D. (1998). Earthworms: Nature's Gift for Utilization of Organic Wastes. In: *Earthworm Ecology*; St Lucie Press, NY, USA. ISBN 1-884015-74-376.

Katsiamides, A., Stürzenbaum, S.R. (2021). Cryptic speciation and blurred species boundaries of the earthworm: a challenge for soil-based toxicological risk assessments. *Comparative Biochemistry and Physiology - Part C.* **239;** 108880. https://doi.org/ 10.1016/j.cbpc.2020.108880.

Minamiya, Y. (2021). 日本産ミミズ大図鑑. Online: https://japanese-mimizu.jimdo.com/; web.archive.org/web/20160404065026/http://japanese-mimizu.jimdo.com:80/.

Rosa, D. (1886). Note sui lombrici del Veneto. *Atti Reale Ist Veneto Sci.* **4**: 673–687.

Rosa, D. (1886). Note sui Lombrici del Veneto (Riassunto), *Bollettino dei musei di zoologia ed anatomia comparata della Torino.* **1(3)**: 1. Online - www.biodiversitylibrary.org/item/43006#page/15/mode/1up .

Sims, R.W., Gerard, B.M. (1999). *Earthworms: Notes for the Identification of British Species.* 4th Edn. Pp. 169. Field Studies Council (31), Montford Bridge, Shrewsbury, UK.

Szederjesi T., Pavlíček T., Márton O., Krízsik V., Csuzdi C. (2019). Integrative taxonomic revision of *Dendrobaena veneta* (Rosa, 1886) *sensu lato* with description of a new species and resurrection of *Dendrobaena succinta* (Rosa, 1905) (Megadrili: Lumbricidae), *Journal of Natural History*, **53**: 5-6, 301-314, DOI: 10.1080/00222933.2019.1593537.